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Elimination of *Lactobacillus plantarum* and achievement of shelf stable model salad dressing by pilot scale pulsed electric fields combined with mild heat

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Abstract

Model salad dressing inoculated with *Lactobacillus plantarum* 8014 was subjected to pulsed electric fields (PEF)-only processing and PEF followed by a mild heat treatment. More than 7 log inactivation was achieved by using PEF-only processing at 34 kV/cm for 45.7 μs with minimal heat contribution. Samples for shelf life evaluation were aseptically packed in 4-oz HIPS/EVOH/PE cups using a Benco® system. The PEF-treated samples were stable at 4 °C for the 8-week evaluation period. However, *L. plantarum* in the samples treated with PEF-only grew up to more than 10^9 cfu/ml in 1 week at room temperature. PEF processing at 31.8 kV/cm for 45 μs followed by a mild heat processing at 67.2~73.6 °C for 24 s resulted in a shelf stable product with an initial *L. plantarum* load of 3.7×10^3 cfu/ml. No *L. plantarum* 8014 recovered in the model salad dressing at room temperature for at least 1 year.

Industrial relevance: Pulsed electric field processing is on the verge of entering industrial scale processing. Consequently pilot scale data are essential for process scale up purposes. Increasing evidence is currently occumulated that PEF in conjunction with moderate heat treatment is not only an attractive minimal processing alternative but also interesting in terms of energy efficiency and low environmental impacts for pasteurization of foods. This paper is one example for such process development for food materials with low (10^3 cfu/ml) initial microbial counts.

1. Introduction

Lactobacillus plantarum is a heterofermentative lactic acid bacterium with %G+C of 45 (Jay 1996) and is widely used for production of pickles, olives, sauerkraut, sausage, Ogi and Gari. *L. plantarum* can grow at pH as low as 3.34. While it is intensively reported probiotic (Goossens et al., 2003; Grangette et al., 2001; Ikenaga, Yamahira, Nachi, Toba, & Okamatu, 2002; Mangell et al., 2002; Mangiante et al., 2001; Marlene, Johansson, & Inga, 2003; Michil & Abernathy, 2002; Pathmakanthan, Li, Cowie, & Hawkey, 2004; Slavka et al., 2004; Suskovic, Kos, Motosic, & Maric, 1997; von Bueltzingsloewen, Adlerberth, Wold, Dahlen & Jontell, 2003) and effective in suppressing the growth of pathogenic and spoilage microorganisms by secreting bacteriocin (Gonzalez, Arca, Mayo, & Suarez, 1994; Ha, Cha, & Han, 1994; Kato et al., 1994; Kim, Ha, & Ray, 1991; Navarro, Zarazaga, Saenz, Ruiz-Larrea, & Torres, 2000; Nissen-Meyer, Larsen, Sletten, Daeschel, & Nes, 1993;) in food, *L. plantarum* was also responsible for the spoilage of many food products such as sauerkraut (Prescott & Dunn, 1959), orange juice (Hays & Riester, 1952), wine (Jay, 1996), beer, mayonnaise and salad dressing (Kurtzman, Rogers, & Hesseltine, 1971). Rapid growth of *L. plantarum* and *L. cucumeris* causes spoilage of sauerkraut, called slimy kraut, particularly at elevated temperatures (Prescott & Dunn, 1959). Malo-lactic fermentation is a spoilage condition of importance in wines. Malic and tartaric acids

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are two of the predominant organic acids in grape juice must and wines. In malo-lactic fermentation, *L. plantarum* decomposes tartaric acid into lactic acid, acetic acid and CO_2 and, as a result, reduces the acidity and changes the flavor of wine. Hays and Reister (1952) investigated samples of spoiled orange juice and found that *L. plantarum* and several other lactic acid bacteria are responsible for the development of a vinegary to buttermilk off-odor with an accompanying off-flavor. *L. plantarum* was also isolated from spoiled salad dressing and mayonnaise (Kurtzman et al., 1971) and considered as one of the causes for flavor change and gas production in the spoiled samples. Elimination of *L. plantarum* is an indicator for quality assurance and shelf life extension of many high acid foods.

Pulsed electric field pasteurization which does not significantly impair the major attributes of the final food products is an emerging nonthermal technology alternative to thermal processing (Bendicho, Barbosa-Canovas, & Martin, 2002; Yeom, Streaker, Zhang, & Min, 2000; Qin, Pothakamury, Barbosa-Canovas, & Swanson, 1996). Microbial inactivation efficacy of PEF was illustrated by inactivating Lactobacilli and yeast (Evrendilek, Li, Dantzer, & Zhang, 2004), *E. coli* (Li & Zhang, 2004; Zhang, Qin, Barbosa-Canovas, & Swanson, 1994), *Lactobacillus brevis* (Jayaram, Castle, & Pargaritis, 1992), *Listeria* (Reina, Jin, Zhang, & Yousef, 1998), and *E. coli* O157:H7 (Evrendilek, Zhang, & Richter, 1999). The inactivation of microorganisms by PEF is due primarily to the high intensity of the electric fields (Zhang, Molsalve-Gonzalez, Barbosa-Canovas, & Swanson, 1994). The microbial inactivation effect of PEF is described as cellular membrane dielectric breakdown when the trans-membrane potential is higher than the critical value of 1 V (Zimmermann, 1986). PEF inactivates more than 5 logs of the natural flora in the enriched soymilk and causes no significant loss in either immunoactivity (Li, Zhang, Lee, & Pham, 2003) or secondary structure (Li, Bomser, & Zhang, 2005) of bovine IgG. Inactivation of enzymes by PEF (Yang, Li & Zhang, 2004) also helps to assure the shelf life of final food products.

PEF processing may be considered as PEF-only or in combination with other hurdles. PEF-only treatment is effective in extending microbial shelf life at refrigerated temperature. However, PEF-only treatment cannot achieve shelf stable food products at room temperature. Efforts have been made to combine PEF with other treatments, such as addition of nisin (Calderon-Miranda, Barbosa-Canovas, & Swanson, 1999), with nisin and lowered water activity (Terebiznik, Jagus, Cerrutti, de Huergo, & Pilosof, 2002), and with high pressure processing (Spilimbergo, Dehghani, Bertucco, & Foster, 2003) to extend the shelf life of products. PEF treatment at elevated temperature of 35~65 °C showed improved efficacy for inactivation of *E. coli* in apple juice and the energy consumption to achieve same level of microbial inactivation could be reduced from over 100 kJ/kg to less than 40 kJ/kg (Heinz, Toepfl & Knorr, 2003). However, attempts to achieve product shelf stability using PEF combined with other technologies are so far not successful. There is a lack of reports regarding the approach of using PEF combined with mild heat to achieve product shelf stability. Although the synergistic effect in microbial inactivation between PEF and heat with a pre-PEF treatment was suspected based on the data using bench scale PEF operations (Evrendilek & Zhang, 2003; Lado, Bomser, Dunne, & Yousef, 2004), little evidence has been reported using formulated food products in a pilot plant or commercial production PEF system while products are packaged aseptically.

The objectives of the study were 1) to investigate the inactivation effect of PEF-only and a combination process of PEF followed by a mild heat treatment against *L. plantarum* and 2) to evaluate the feasibility of using a combination process of PEF followed by a mild heat treatment to achieve shelf stable products at room temperature, and 3) to study the effect of different processing on pH, electric conductivity, viscosity and color of samples when stored at room temperature.

2. Materials and methods

2.1. Materials

2.1.1. Model ranch salad dressing

The model ranch salad dressing used in this study was prepared with modified corn starch, cane sugar, whey protein powder, citric acid powder, table salt and tap water (Table 1). All the ingredients used are food grade products purchased from local grocery stores and food ingredient distributors. The prepared model salad dressing had a pH of 4.3 and an electric conductivity of 0.62 S/m at room temperature. The flow behavior of the model was similar to that of ranch salad dressing in conductivity and viscosity.

2.1.2. L. plantarum inoculum

The *L. plantarum* (ATCC 8014) was activated from deep frozen storage MRS (Man, Rogosa and Sharpe) plus glycol

Table 1
Composition of the model salad dressing*

Ingredients	Amount used (g)
Tap water	90,000
Sucrose	6261
Whey protein powder	1997
Citric acid powder	43
Table salt	272
Modified corn starch	6261

* All ingredients used are food grade.

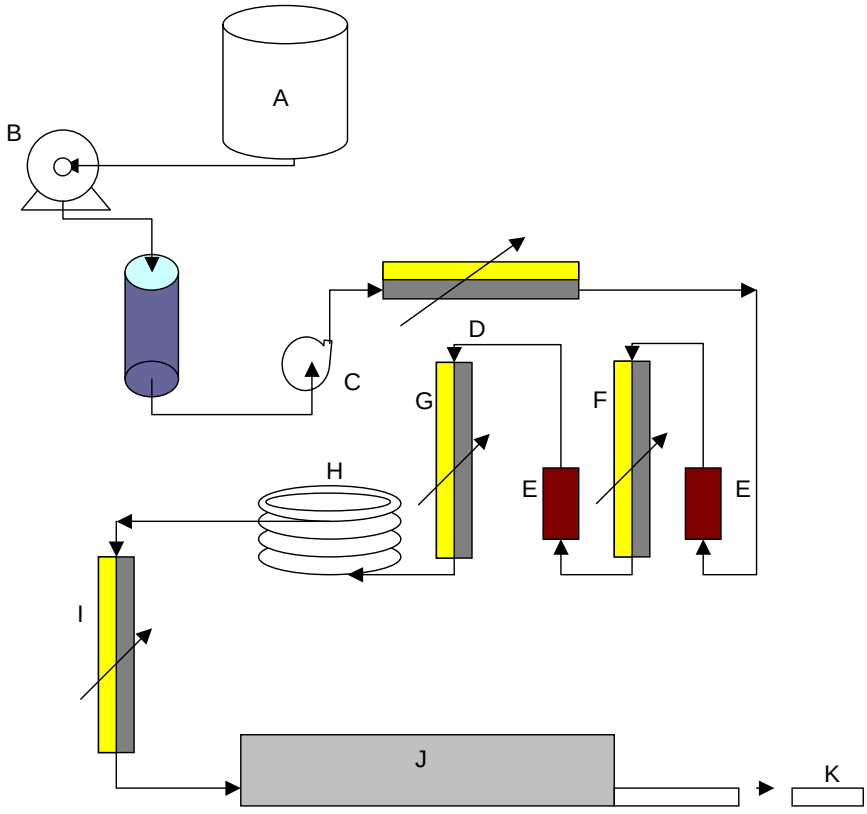

Fig. 1. Schematic diagram for PEF/mild heat combinational processing of model salad dressing. Denotes of the graph: A. product source tank; B. deaerator; C. feeding pump; D. heat exchanger; E. PEF treatment chamber set (two chambers in each set); F. heat exchanger; G. heat exchanger; H. holding tube; I. heat exchanger; J. Benco aseptic packaging machine; K. labeling and storage.

broth culture into MRS broth at 35±2 °C with multiple transitions before the preparation of working culture. Working culture was prepared with 1% inoculation level in MRS broth and then incubated at 37 °C for 8 h. The cells used for inoculation of the model salad dressing were at their log growth phase.

2.1.3. Test sample

The model salad dressing sample was prepared by inoculating approximately 10^9 (for PEF inactivation tests) and 10^3 colony forming units (cfu)/ml (for shelf life evaluation) of *L. plantarum* lag-phase cells into the model dressing. Inoculation took place before the addition of the

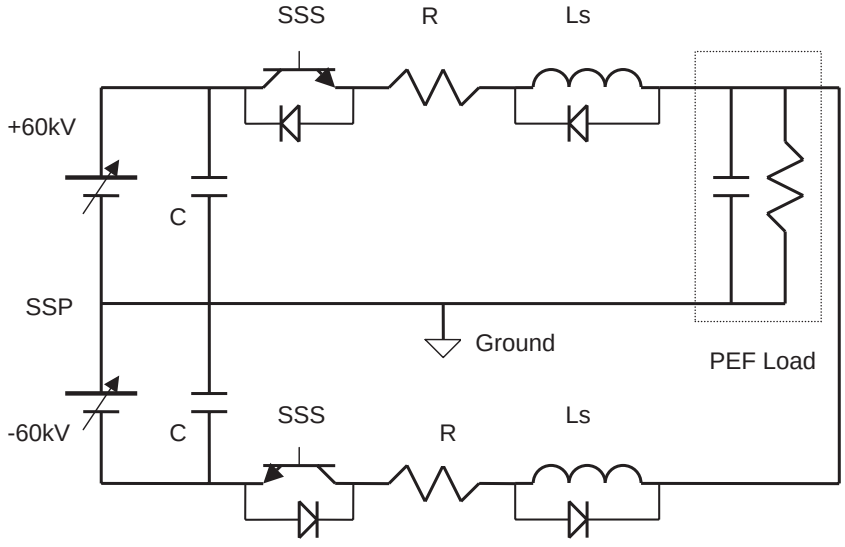

Fig. 2. Circuit block diagram of OSU-6 commercial scale pulse generator, with a maximum peak voltage output of 60 kV.

Table 2
Conditions for PEF-only processing of the inoculated model salad dressing with *L. plantarum* 8014

Flow rate	125 L/h
System pressure	0.34 MPa
Deaerator vacuum	97.5 mmHg
T at hopper	13 °C
Waveform	Bipolar, square
Electric field strength	0~35 kV/cm
Total treatment time	45.7 μs
$T_{\text{holding tube in}}$	37 °C
$T_{\text{holding tube out}}$	37 °C
$T_{\text{cooled to after treatment}}$	37 °C
Holding time	24 s

modified corn starch. The sample was stored at 4 °C overnight for next day PEF processing.

2.2. Methods

2.2.1. PEF treatments

PEF treatments of the inoculated salad dressing were performed with a modified pilot scale OSU-2 fluid handling systems (Fig. 1) that integrated with a commercial scale OSU-6 pulse generator (Diversified Technologies, Bedford, MA) (Fig. 2). Four co-field flow PEF treatment chambers (gap distance 1.27 cm and inner diameter 0.635 cm) were used in series along the flow direction. PEF chambers were installed vertically to achieve high performance. An in-line versator was used at 740~750 mmHg vacuum before PEF treatment to remove air trapped in the model salad dressing to eliminate electric arcs. The flow chart of the PEF and/or mild heat processing is illustrated in Fig. 1. Heat exchanger D was used to adjust sample inlet temperature (measured at the entrance of first PEF treatment chamber) to the designed level. During PEF-only processing, heat exchanger F was operated to cool the sample temperature down to approximately the same initial temperature as the inlet of first PEF treatment chamber. Heat exchange G cooled the temperature of the processed samples down to approximately 37 °C prior to aseptic packaging. Heat exchanger I was turned off during PEF-only processing. The flow rate of the fluid handling system was fixed at 125 L/h and the system back pressure was maintained in the range of 0.24–0.38 MPa. The PEF treatment conditions and fluid handling system parameters are summarized in Table 2. Bipolar square pulses with a pulse width of 1.7 μs were applied for all treatments.

2.2.2. Combination process of PEF and mild heat

The combination process of PEF treatment followed by a heat treatment of the high acid food is illustrated in Fig. 1. The process is similar to that for the PEF-only process except, during the combination process: 1. heat exchanger F is turned off; 2. heat exchanger G is used to further increase the sample temperature after PEF treatment to the designated temperature for the heat treatment; 3. heat exchanger I is on to cool the processed sample down to approximately 37 °C prior to aseptic packaging. The holding time of samples in the holding tube is flow rate dependent. During this study, the sample flow rate was fixed at 125 L/h and the holding time of the sample in the holding tube was 24 s for all the samples. The processing conditions for the combination processing of PEF followed by a mild heat treatment are summarized in Table 3.

2.2.3. Package of the PEF-treated model salad dressing samples

PEF-treated model salad dressing samples were packed aseptically in 4-oz thermally formed cups (base material HIPS/EVOH/PE, cup wall thickness of 0.28 mm) with an in-line Benco® ASPAK/2 aseptic packaging system (Benco Pack S.p.A., Piacenza, Italy). Sample name, date and processing conditions were labeled right after sealing.

After the collection of PEF-treated samples, control samples that were not subjected to either PEF treatment or heat treatment but pumped through the fluid handling system at same flow conditions were collected. Sample name, processing date and conditions were labeled immediately on the cups.

2.2.4. Storage of samples for microbial stability evaluation

The control samples either collected at the product hopper or packaged by the aseptic package machine without PEF treatments, and PEF-treated model salad dressing samples were stored at room temperature.

2.2.5. Routine check for pop-ups of the packed samples

Samples were visually checked at various time intervals for pop-ups among the all samples. Cups with obvious gas production and accumulation, reflected by the pop-up of the cup lids due to the positive pressure build-up inside the cups, were considered as spoiled samples and a failure of microbial stability.

Table 3
Treatment conditions by PEF followed by a heat treatment on *L. plantarum* 8014 inoculated model salad dressing*

Dates	FR (L/h)	BP (MPa)	Use of versator	E (kV/cm)	τ (μs)	Rep-rate (pps)	Σt (μs)	T_1 (° C)	T_{hi} (° C)	T_{ho} (° C)
06/25	125	0.34	Yes	31.8	1.7	400	45	22.3	73.6	67.2
06/26	125	0.31	Yes	31.2	1.7	400	45	24.2	73.1	66.8

* The retention time of samples in the holding tube was 24 s at 125 L/h flow rate. T_{hi} refers to the inlet temperature of sample in the holding tube. T_{ho} refers to the outlet temperature of sample in the holding tube. Σt presents the total PEF treatment time.

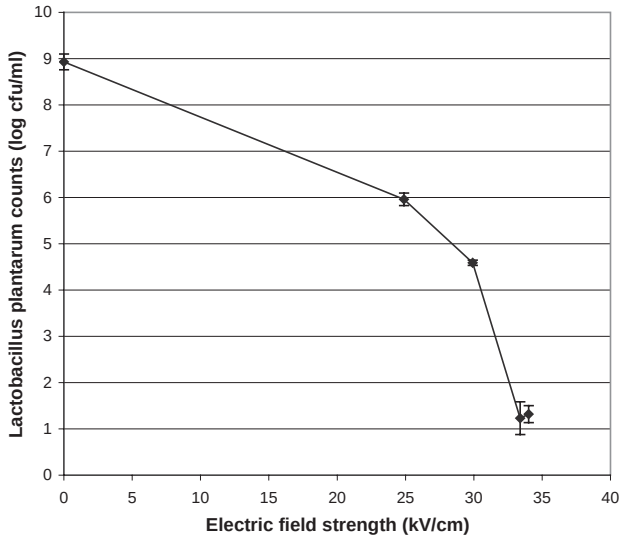

Fig. 3. Inactivation of *Lactobacillus plantarum* by PEF for 45.7 μs with bipolar square pulses and sample inlet temperature of 18 °C and maximum temperature exposure at 47 °C for 3 s.

2.2.6. Enumeration of L. plantarum

Sterile peptone (0.1%) water was used for sample dilution. *L. plantarum* in the samples was enumerated using MRS agar by incubating the surface plated dishes at 37 °C for 48 h. Three dishes were surface plated and incubated for enumeration of *L. plantarum* for each selected dilution and the mean counts and standard deviations are reported.

2.2.7. Experimental design

The PEF inactivation studies against *L. plantarum* in the model salad dressing system were conducted at different electric field strengths for a fixed PEF treatment time of 45.7 μs. Sample inlet temperatures were changed to investigate its influence on the overall inactivation by PEF against *L. plantarum* 8014. Samples treated by PEF treatment alone were packed aseptically in the 4-oz cups and stored in a walk-in refrigerator to evaluate the microbial stability over a 6-week storage period. The tests to evaluate the effect of the PEF and mild heat combined treatment on the inactivation of *L. plantarum* and the shelf stability of the treated samples were carried out at the maximum practical electric field strength for the samples. Thermal hold temperature after PEF treatment was close to 70 °C based on a previous study (not reported) that 70 °C thermal treatment for 2 min maintained acceptable sample sensory characteristics. Different initial *L. plantarum* loads were investigated.

2.2.8. Statistical analysis

Student's tests with tukey multiple comparison were conducted with Minitab 13.31 (Minitab, College Park, PA) at significance level of 0.05. All tests were repeated three times and means were reported with standard deviation.

3. Results and discussion

3.1. Inactivation of L. plantarum in model ranch salad dressing by PEF processing

Inactivation of *L. plantarum* cells by PEF-only treatment is illustrated in Fig. 3. Significant inactivation effects were observed after the PEF treatments ($p<0.01$). More than a 7 log reduction was achieved by PEF treatment at 34 kV/cm for 45.7 μs with a sample temperature of 18 °C and maximum temperature exposure at 47 °C for less than 3 s (2.7 s from 4th chamber to heat exchanger G).

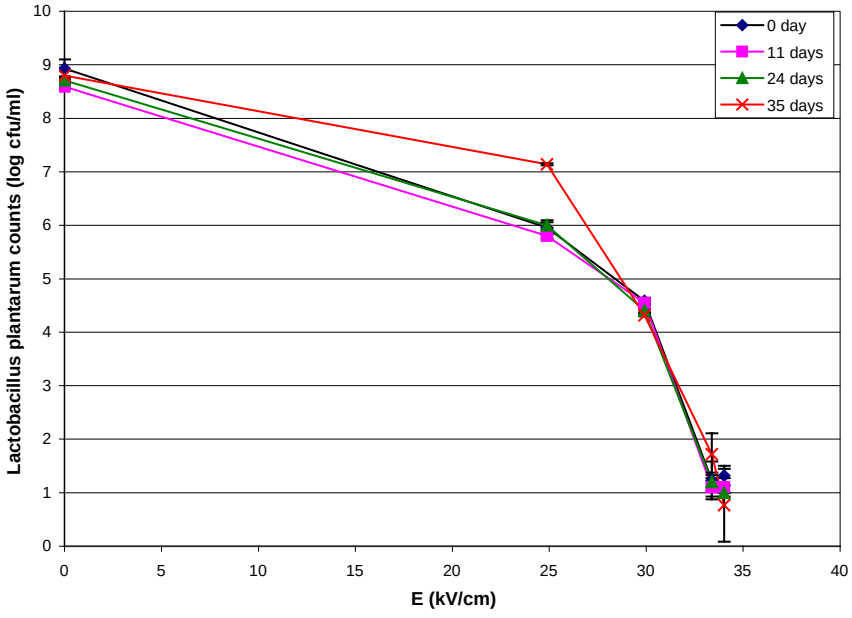

Fig. 4. Inactivation of *Lactibacillus plantarum* 8014 by PEF for 45.7 μs at sample inlet temperature of 18 °C and the microbial shelf stability when stored at 4 °C.

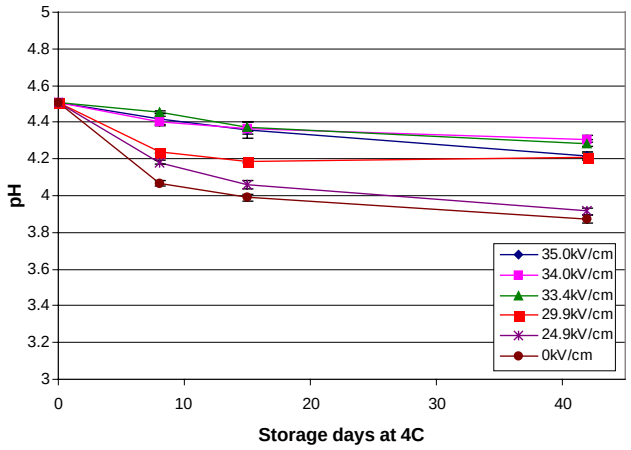

Fig. 5. Effect of PEF treatment on the stability of pH during storage at 4 °C after PEF treatment at different electric field strengths for 45.7 μs with a sample inlet temperature of 18 °C.

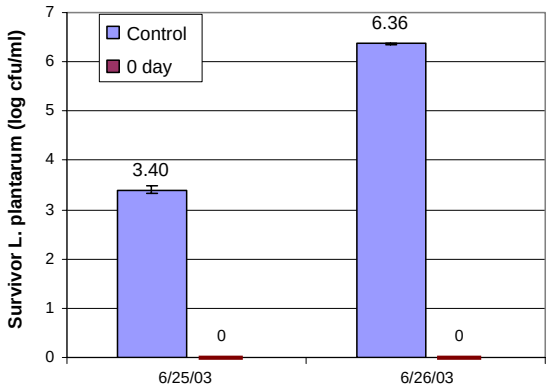

Fig. 6. Inactivation effect of PEF combined with mild heat on *L. plantarum* with different initial microbial load. *X*-axis presents the date when samples were processed and packed with a PEF treatment at 31.2–31.8 kV/cm for 45.7 μs with bipolar square pulses followed by a mild heat treatment at 70 °C for 24 s.

Maximum temperature increases per pair of chambers at 34 kV/cm was 27.9 and 29.3 °C for the first and second pair of chambers, respectively. With the increase of electric field strength, the inactivation of *L. plantarum* 8014 increases with elevated efficiency. The PEF inactivated *L. plantarum* cells did not recover in the model salad dressing during storage at 4 °C for 35 days when the electric field strength was higher than 30 kV/cm (Fig. 4). However, at 25 kV/cm electric field strength treated for 45.7 μs, after storage at 4 °C for 35 days, significant recovery of *L. plantarum* cells was observed (p<0.01). Some of the *L. plantarum* cells treated by PEF at 25 kV/cm were presumably at a sublethal or injury status and recovered when stored at 4 °C for 35 days. The recovery may take place more rapidly when the sample is stored at a higher temperature. Samples treated at 34 kV/cm for 45.7 μs spoiled in 1 week and the *L. plantarum* count reached 10^9 cfu/ml in 2 weeks at room temperature. PEF treatment with minimal heat contribution can significantly extend the microbial shelf life of the inoculated model salad dressing at 4 °C (p<0.01). Similarly, *L. plantarum* inoculated in sterile milk recovered after a heat treatment at 72 °C holding for 90 s and stored at 7 °C for 20 days (De Angelis et al., 2004). It is very unlikely that either PEF-only or heat treatment below 72 °C for 90 s will eliminate *L. plantarum* cells.

3.2. Effects of PEF treatment on the stability of pH and electric conductivity

Effects of PEF treatment on stability of pH during a 6-week storage test at 4 °C are illustrated in Fig. 5. No significant change in pH and electric conductivity was observed after PEF treatment from 0 to 34 kV/cm for 45.7 μs (p>0.05). However, significant differences in pH among the samples treated at different electric field strengths ranging from 0 to 34 kV/cm was observed after 1 week of storage at 4 °C (p<0.05). The difference between the two groups of samples treated at 0, 25 kV/cm and those treated at 30, 33.4 and 34 kV/cm increases with extended storage (Fig. 5). The decrease in pH in the group of samples treated at 0 and 25 kV/cm may be due to the recovery and growth of *L. plantarum* cells, which produce lactic acid from carbohydrates and reduce the pH of the medium (Jay, 1996). No statistically significant change in electric conductivity in all the samples was observed during the 6-week storage test at 4 °C.

3.3. Inactivation of L. plantarum cells in model salad dressing by PEF followed by a mild heat treatment

No survival *L. plantarum* cells in the samples treated by PEF at 31.8 kV/cm for 45 μs with a sample inlet temperature of 22.3 °C and followed by a heat process at 67.2 to 73.6 °C (average 70.4 °C) for 24 s was detected with an initial *L.*

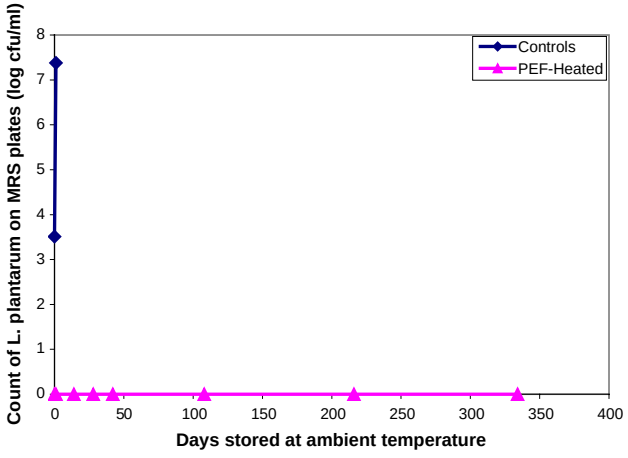

Fig. 7. Microbial shelf life of PEF-treated and control model salad dressing samples packed aseptically in 4-oz multilayer in-line thermal formed cups when stored at room temperature (23–25 °C). "0" on *y*-axis represents the fact that no colony was developed on the plates after being incubated at 37 °C for 48 h when using 0.1 ml of non-diluted sample for surface plating (<10 fu/ml).

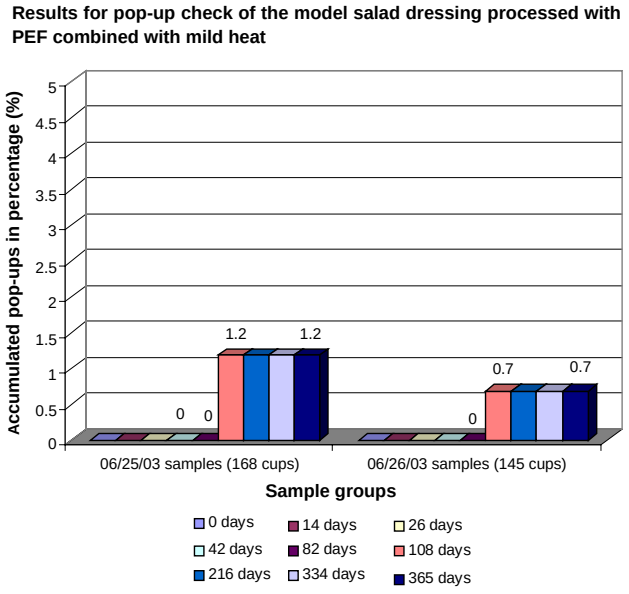

Fig. 8. Percentage pop-ups among the PEF-treated and aseptically packed model salad dressing in 4-oz thermally in-line formed multilayer cups at room temperature.

plantarum load of 2.5×10^3 cfu/ml (Fig. 6). The samples treated by PEF combined with mild heat treatment had a *L. plantarum* count of less than 1 cfu/ml (est.). At similar combination treatment conditions, increase in the initial *L. plantarum* load to 2.3×10^6 cfu/ml (Fig. 6) also resulted in a *L. plantarum* plate count of less than 1/ml (est.). More than 6 log reduction in *L. plantarum* population was observed by the combination treatment of PEF and mild heat. No detectable recovery of *L. plantarum* cells was observed after the combination treatment of PEF and mild heat during storage at room temperature for at least 11 months (Fig. 7). As a comparison, *L. plantarum* in the samples that were treated using a batch heat treatment at 72 °C for 2 min with an initial count of 10^6 cfu/ml resulted in more than 5 log reduction when measured right after treatment. However, *L. plantarum* cells recovered and grew to a level of higher than 10^9 cfu/ml in 2 weeks at room temperature. This phenomenon agreed well with a previous report (De Angelis et al., 2004) conducted with sterile milk treated at 72 °C for 90 s then followed by storage at 7 °C for 20 days. The authors also reported the *D*-value of *L. plantarum* in sterile milk was 14.7 s at 72 °C and 7.14 s at 75 °C and *z*-value was between 9 and 20 °C. The combination of PEF and mild heat showed significantly higher inactivation efficacy against *L. plantarum* cells in the model salad dressing. Additionally, the combination treatment of PEF and mild heat eliminated the *L. plantarum* cells inoculated in the model salad dressing without detectable recovery during storage at room temperature. Based on the results of inactivation studies using PEF, heat and the combination treatments, we conclude that combination treatments using PEF and mild heat have significant synergistic effects against *L. plantarum*.

3.4. Extension of microbial shelf life of the inoculated model salad dressing and achievement of shelf stable products at room temperature

The room temperature microbial stability of the inoculated model salad dressing was significantly improved (Fig. 7) after the PEF + mild heat treatments ($p<0.01$). No *L. plantarum* colony was observed in non-diluted PEF-treated samples on the MRS agar plates at 37 °C after both 48 h and extended incubation of 96 h. The samples treated by PEF at 31.8 kV/cm for 45.7 μs followed by mild heat at 67.2~73.6 °C for 24 s were microbiologically shelf stable for at least 11 months. The evaluation of microbiological stability of the treated samples at room temperature is still ongoing.

The pop-up check results (Fig. 8) match well with the *L. plantarum* counts. The percentage of pop-ups was maintained at 0% for the first 82 days of storage at room temperature (Fig. 8). After 108 days of storage at room temperature, 2 cups popped up among the 168 cups packed on June 25, 2003 and resulted in an accumulated percentage of pop-ups of 1.2%, which was stable throughout the rest of the storage test at room temperature for 1 year. The 145 samples packed on June 26, 2003 showed one cup popped-up after 82 days storage at room temperature and the accumulated percentage of pop-ups maintained stable at 0.7% throughout the rest of the 1-year storage test. The two batches of packed samples were stored at room temperature under further investigation and showed no evidence of gas production to cause more pop-ups after 13 months. We can conclude that PEF treatment followed by a mild heat treatment at 67.2~73.6 °C for 24 s is able to achieve a room temperature shelf stability when processing model salad dressing with *L. plantarum* inoculation level less than 10^6 cfu/ml.

4. Conclusions

PEF-only treatment at 34 kV/cm for 45.7 μs results in more than 7 log reduction in *L. plantarum* and significantly extends the microbial shelf life of the treated model salad dressing when stored at refrigerated conditions. No significant change in electric conductivity was observed before and after the PEF treatment. However, significant recovery of *L. plantarum* cells was observed after PEF treatment during storage at 4 °C for 35 days when treated at 25 kV/cm. There is a significant synergistic effect between PEF and a subsequent mild heat treatment in the inactivation of *L. plantarum* 8014 in the model salad dressing system. PEF + mild heat processing at 31.8 kV/cm for 45.7 μs followed by a heat treatment at 67.2~73.6 °C for 24 s was shown to eliminate *L. plantarum* 8014 in model salad dressing to achieve a room temperature shelf stability longer than 11

months. Less than 1 cfu/ml *L. plantarum* in the treated samples was observed after 11 months of storage at room temperature.

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